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Amphetamine (Benzedrine) Sulfate upon Higher Nervous Activity Compared with Alcohol. II. Human Experiments. NATHANIEL FINKELSTEIN, E. BRYCE ALPERN AND W. HORSLEY GANTT 61

Clinical and Pathological Findings in Aortic Atresia or Marked Hypoplasia of the Aorta at Its Base. HELEN B. TAUSSIG, M.D. 75

The Methylation of Nicotinamide. A Simple and Practical Test for Liver Function (A Preliminary Note). VICTOR A. NAJJAR, ROWENA SIDBURY HALL AND CAROLYN C. DEAL 83

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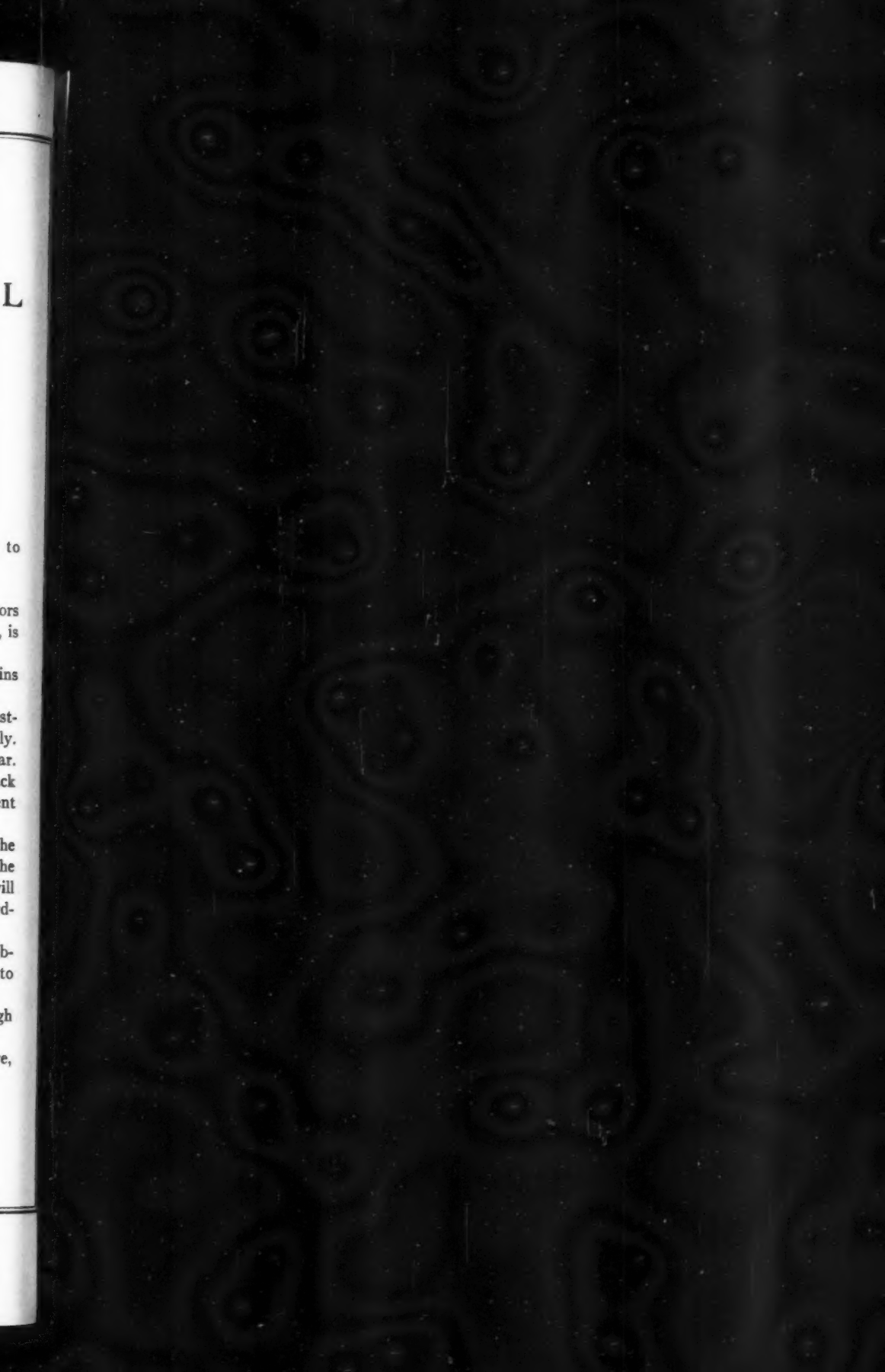

AMPHETAMINE (BENZEDRINE) SULFATE UPON HIGHER NERVOUS ACTIVITY COMPARED WITH ALCOHOL

II. Human Experiments

NATHANIEL FINKELSTEIN, E. BRYCE ALPERN AND W. HORSLEY GANTT
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The experimental work on the effect of amphetamine (benzedrine)[1] sulfate on the performance of human beings has been handicapped by the individual variations in response and by the difficulty in selecting a suitable test of performance. Although the drug is widely used to improve performance, the experimental evidence that it does so is contradictory. Previous reports (Ivy and Krasno (1)) showed that performance, in general, appeared to be improved after amphetamine sulfate, particularly if fatigue and boredom played a rôle in the situation (see Table 1). In a previous paper (2) concerning the effect of this drug on the higher nervous system of dogs, in which the conditional reflex[2] (motor, secretory, and autonomic) was employed as a measure of performance, we showed that 1 mg. per kg. of amphetamine sulfate has a stimulatory action on the cerebral cortex, but there was a concomitant loss of differentiation. It seemed advisable to carry out a similar study with human beings using a dosage more nearly approximating the usual therapeutic one (0.10–0.40 mg. per kg.).

In normal human subjects measurement of cr activity has not been as well standardized as it has in the study of various animals as the dog, the rat, and the sheep. A simple procedure has been described by Gantt (15, 16) using positive and negative conditional stimuli and an electrical shock for the unconditional stimulus. All normal individuals were able to make the simple differentiation between a few stimuli, as could most psychotics (excepting severely deteriorated-schizophrenics, Korsakovs, etc.), but individuals with organic brain damage failed. For work with normal subjects this test was not a sufficiently severe test of ability to form crs, so that a more complex one had to be employed.

[1] The amphetamine sulfate and the placebos used in this experiment were supplied through the courtesy of Smith, Kline and French Co., Philadelphia.

[2] Conditional (conditioned) reflex is abbreviated as *cr*, and unconditional reflex as *UR*.

METHOD

The subjects of this study were medical students and student nurses between the ages of 18 and 28. They were instructed to take no drugs, coffee or tea before each experiment. A fairly standardized

TABLE 1

AUTHOR	NO. OF SUBJECTS	DOSAGE	TEST	RESULT
		mg.		
Molitch and Sullivan (2)	96 boys	10–20	Stanford Test	Improvement
Molitch and Eccles (3)	93 boys	10–30	Memory for designs, Intelligence tests	Improvement
Bradley (4)	30 children	20	School performance	50% improvement
McNamara and Miller (5)	10 students	20	Multiplication problems	No improvement
Barmack (6, 7)	36 students	10	Addition problems	Improvement, Boredom decreased
Carl and Turner (8)	143 adults	10–30	Psychometric tests	Improved speech and accuracy—especially with increased periods of work
Kleitman (9)		10?	"Blocking" during continuous testing	Faster performance, Fewer errors
Csinady and Dirner (10)	5 subjects	20	Mathematical problems, Foreign vocabulary, Bourden test	No change, Rate of learning increased, Improvement.
Bhansen, Jacobsen and Thesleff (11)	100 adults	10–20	Reaction time, accuracy hand movements	No improvement
Wollstein (12)	100?	15	Attention and missing word test	No change
Thornton, Holck and Smith (13)	3 adults	20	Psychomotor, Reasoning and disarranged words	No consistent change

procedure was carried out with each subject, the entire study taking place in one room with as little distraction as possible. The test for each subject consisted of two parts: 1) the resting blood pressure, resting respiratory rate, resting pulse rate, threshold of sensitivity to an electrical shock, motor cr performance, performance with a number sequence test, and acuity of hearing (with only a few subjects). 2) The second part of each experiment began fifteen minutes after com-

pletion of the above tests and consisted simply in repeating the tests. Any effect of adaptation to the situation by repetition could be estimated from this set of controls.

The control series were a group of 17 subjects used in both parts of the test. The average difference (and the standard deviation) between the measurements obtained in the two parts were calculated. The amphetamine series consisted of a group of 19 subjects who received a placebo, similar in taste and appearance to amphetamine sulfate, before the first part of the test, and then received either 10–15 or 20–30 mgs. of amphetamine sulfate before the second part, the average difference again being calculated. The measurements during the second part of the test were made during the time of maximal effect of the drug. To ensure adequate controls, only one-fourth of the measurements were made while the subjects were under the influence of the drug.

The blood pressure was measured by a mercury manometer and stethoscope, the pulse rate by palpating the radial pulse under resting conditions, and the respiratory rate by a pneumograph recording on a kymograph. Hearing acuity was measured in a few subjects with an audiometer. All these records were taken by the same experimenter when the subject was quiet.

The apparatus used for forming and testing the motor crs consisted of two boxes placed in front of the subject and examiner respectively (15). On the surface of the subject's box were two brass electrodes upon which he placed his hand and through which he received the electrical shock. There were five stimuli on the subject's board: bell, buzzer, and green, red, and white lights. There was also a cutoff switch, which, when closed by the subject prevented his receiving the shock. The operating box, which was kept from the view of the subject, contained switches which controlled the auditory and visual stimuli, a switch to give the shock, and an inductorium which graded the intensity of the shock. During the examination, the onset of the conditional stimulus and of the shock, the primitive and integrated crs seen in the two hands and the respiration were recorded on a kymograph (see Figures 1, 2).

The threshold of sensation of each hand was measured by the average of three readings of the least strength of shock perceived. The unconditional motor stimulus was then set by the rheostat at an

intensity which would cause a definite withdrawl of the hand, and this intensity was used during the formation of the conditional reflexes.

In each test, eight conditional stimuli were given by using one or a combination of two of the lights or sounds. Five of these were positive stimuli, i.e., reinforced after 3–5 seconds by the giving of the shock,

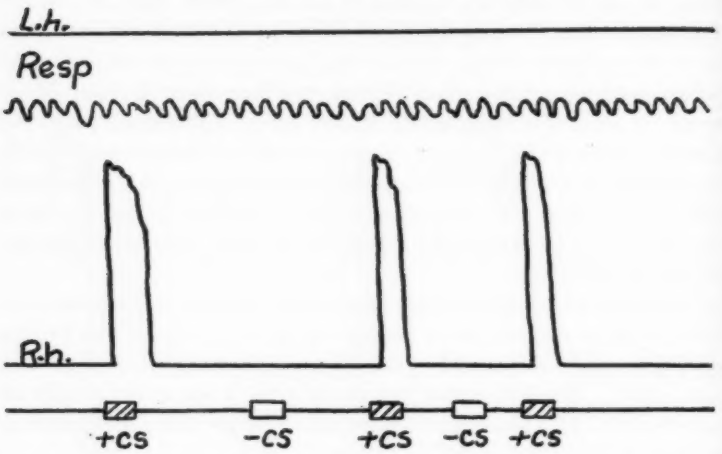

FIG. 1. CONTROL CONDITIONAL REFLEX RECORD IN J. W. T. R., 2 MAY 1944

L.h. (top line) = left hand (passive hand on electrodes);
Resp. (2d line) = respiration;
R.h. (3rd line) = integrated conditional reflexes in right hand;
Cs (bottom line) = excitatory (shaded squares) and inhibitory (white squares) conditional signals (during 3 seconds).
US (bottom line) = unconditional stimulus, i.e., shock (black square).

and three were negative, i.e., not reinforced. The first series of eight consecutive stimuli was presented without instruction save that the subject place his hand on the brass plates and observe very carefully what occurs. After this series had been completed, the subject was told that he could avoid the shock if he pushed the cutoff switch when he expected a shock. Then two further series of eight were given, changing the order of the conditional stimuli so that there was no fixed pattern. From the kymographic records (Figure 1) was obtained the percentage of correct responses to a positive stimulus (throwing of

the cutoff switch or pressing a bulb) and the percentage of correct responses to a negative stimulus (no throwing of the cutoff switch), the respiratory change with each stimulus, and the latent period of the throwing of the cutoff switch (motor cr).[3]

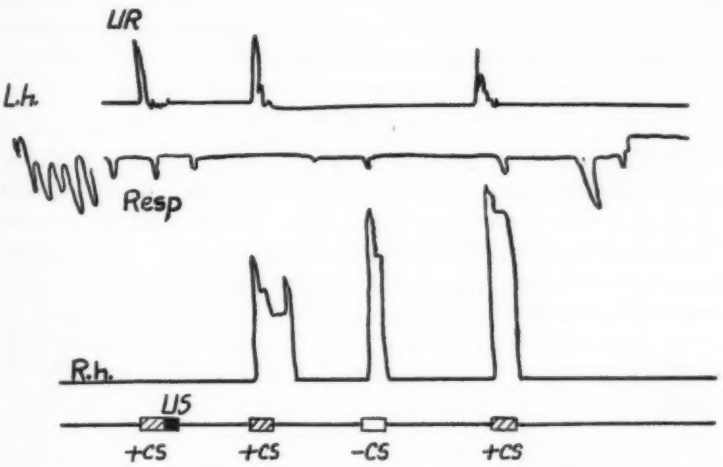

FIG. 2. ALCOHOL CONDITIONAL REFLEX RECORD — ABOUT 90 MINUTES AFTER INGESTION OF 2 CC. ALCOHOL/KG. IN J. W. T. R., 29 APRIL, 1944

See Fig. 1 for legend. Note the primitive type of conditional reflexes in top line, the Cheyne-Stokes respiration compared with the regular respiration of Fig. 1, the difference in pattern of the integrated movements in the right hand (R.h.) compared with Fig. 1, the failure at one time to react to the excitatory conditional stimulus and at another time the reaction to an inhibitory conditional stimulus.

As a parallel test, a set of number series completion tests from the Army Alpha test was given (5). Each test consisted of fifteen lines,

[3] The conditional stimuli were given at about 20–60 second intervals. The intelligent subjects had several ways of avoiding the shock. Some memorized the stimuli—these people impressed us as slow but stable. Others tried an association of one sort or another, e.g., the more experienced realized there were only 3 negative stimuli as compared with 5 positive; they memorized only the 3 negative and turned the switch to all others. Others tried to place the stimuli into categories and make various associations; these had the most difficulty. A few after getting the shock decided to take no more chances and pushed the switch to all stimuli—a kind of "throwing up the sponge."

having a series of numbers terminating in a blank (e.g., 1, 3, 5, 7, 9, –), the subject filling in the number which followed in sequence. The time taken to complete the test and the accuracy were determined. The first test was given when the subject entered the laboratory and the result discarded. The second test was given during the first part of a trial, and the third test during the second part of a trial, the difference between these two tests being calculated.

RESULTS

The results in this experiment (Tables 2 and 3) consisted of a comparison between the determinations of a control and of an amphetamine series, each determination consisting of the average difference between the measurements obtained in the two parts of a trial. For example, if in the control series a represents the pulse rate in the first part of the trial, and a' represents the pulse rate in the second part of the trial, and in the amphetamine series b represents the pulse rate in the first part and b' the pulse rate in the second part, the calculations were made as follows:

$$\frac{(a' - a)}{n} = \text{average difference in control series}$$

$$\frac{(b' - b)}{n} = \text{average difference in amphetamine series}$$

average difference in amphetamine series − average difference in the control series = difference between series.

To determine whether the difference between the two series was statistically significant all the amphetamine measurements regardless of dose were grouped to increase the size of the sample. When there was less than one out of 20 chances of any difference between the series being due to a chance variation, the result was considered significant and therefore an effect of the drug.

The effect of benzedrine sulfate in raising *blood pressure* is well known (21). In these subjects there was a rise of systolic pressure of 8 mms. with 10–15 mgs. and 16 mms. with 20–30 mgs. benzedrine sulfate.

TABLE 2

Summary of results

A tabulation of the average differences obtained in the control and in the amphetamine series. All amphetamine readings have been averaged regardless of dose in order to determine statistical signicfiance.

MEASUREMENT OF	AV. DIFF. IN CONTROL SER.	AV. DIFF. IN AMPH. SERIES	DIFF. BET. SERIES	ST. DEV. CONT. (σ) SERIES	ST. DEV. AMPH. (σ) SERIES	NO. OF CONT.	NO. OF AMPH.	SIGNIF. RATIO
Blood press.								
systolic.....	−4.0 mm.	+9.6 mm.	+13.6 mm.	4.7	7.3	16	18	6.5*
Diastolic	−3.5	+6.9	+10.4	6.1	7.0	16	18	4.7*
Resp. rate.....	−9.0%	+12.0%	+21.0%	17.0	13.4	16	18	4.0*
Pulse rate								
(beats/min.).	+0.1	−2.6	−2.7	9.3	9.7	19	19	0.3
Sensitivity								
right hand...	−0.21 units	−0.15 units	+0.06 units	0.5	0.4	21	18	0.4
left hand....	−0.34	−0.25	+0.09	0.5	0.6	21	18	0.6
Audiogram	0	0	0					
% cs correct								
positive	+9.0%	+7.5%	−1.5%	17.0	19.2	17	19	0.2
negative.....	−2.0%	+14.0%	+16.0%	22.7	24.0	17	19	2.05*
Latent period								
of cr.....	−8.0%	+8.0%	+16.0%	24.5	26.4	17	19	1.9
Problem test								
duration.....	0.0 sec.	−10.0 sec.	−10.0	17.4	22.1	20	19	1.5
% correct ...	+0.1%	−0.5%	−0.4%	1.4	1.3	20	19	1.0

* When this ratio is greater than 2.0, the chances are less than 1 to 20 that the differ ence is a chance variation.

TABLE 3

Tabulation of statistically significant results due to amphetamine sulfate

MEASUREMENT	10–15 MGS. BENZ.			20–30MGS. BENZ.		
	Av. diff. control series	Av. diff. amphetamine series	Diff. between series	Av. diff. control series	Av. diff. amphetamine series	Diff. between series
Blood pressure						
systolic....	−4.0 mm.	+4.4 mm.	+8.4 mm.	−4.0 mm.	+12.2 mm.	+16.2
diastolic...	−3.6	+2.9	+6.5	−3.6	+8.9	+12.5
Resp. rate...	−9.0%	+14.0%	+23.0%	+9.0%	−11.0%	−20.0%
% negative cr						
correct....	−7.0%	+7.0%	+14.0%	−7.0%	+18.5%	+25.5%

There was likewise a rise in diastolic pressure of 6 mms. and 12 mms. respectively. This small elevation in blood pressure was consistent enough in each individual to be used as an indication that the subject was under the influence of the drug.

There was an increase in the resting *respiratory rate* of 23 per cent after 10–15 mgs. and 20 per cent after 20–30 mgs. of benzedrine sulfate. The resting pulse rate was not altered by the drug. The threshold *sensitivity* of the palmar surface of the hands to an electrical shock and the acuity of hearing at all pitches was unchanged.

The *cr activity* was measured by the change in the percentage of correct responses to the series of positive conditional stimuli and in the percentage of correct responses to the set of negative conditional stimuli. The drug had no effect on the response to a positive conditional stimulus, either in 10–15 or 20–30 mg. dosage, but it increased the number of correct responses to a negative conditional stimulus by 14 per cent after 10–15 mgs. and by 25 per cent after 20–30 mgs.

The *latent period* of the motor response, which in similar experiments with dogs was influenced variously, showed no change. The latent period is a reciprocal measure of the rapidity of response and tends to be decreased when the motor cr activity is stimulated.

The respiratory cr was present in both the control and benzedrine series. This reflex consisted of an increase in the respiratory rate in the interval between the conditional stimulus and the unconditional stimulus, and was greater with a positive conditional stimulus than it was with a negative, showing that differentiation was present. There was no change in this reflex after amphetamine.

There was no improvement or impairment of the ability to perform the number series test, no change occurring either in the accuracy or in the speed.

In all of the subjects there were objective and subjective mood changes. Usually the subjects were more talkative and stated that they felt more wideawake and cheerful. Two subjects who were given the drug early in the day, before they had eaten breakfast, suffered from nausea and anorexia. One subject complained of frequency of urination during the night after the test. Another was relieved of painful menstrual uterine cramps.

EFFECT OF ALCOHOL

In order to demonstrate that the cr method used was sensitive enough in humans to pick up the effect of drugs having a pronounced cortical action and to compare its effect with another drug whose action on behavior has been studied, four subjects were tested after the administration of ethyl alcohol. One of us (18) has shown that, in dogs, alcohol increased the latent period of motor responses, decreased the intensity of the conditional reactions, and disturbed the balance between excitation and inhibition.

1.5 to 2.0 cc. per kg. body wt. of ethyl alcohol in the form of whiskey, vermouth, and beer was imbibed by three male graduate students and one nurse, one of whom had previously taken part in an amphetamine sulfate trial, and the usual tests performed including controls either preceding or following alcohol (next day or later). They were tested from 15 minutes to 2 hours after the ingestion of alcohol on an empty stomach. A sample control record is given in fig. 1 and the effect of alcohol in fig. 2. The record (fig. 2) shows the effect of less than the full amount of alcohol given as no records could be taken during the maximum action. Although there were individual variations, all of the subjects showed the following changes.

Threshold of sensitivity. The threshold to tactile perception of a faradic current (lower limit) as well as the upper limit of tolerance to the current was measured. There was a marked decrease in both perception of the least current and the feeling of pain. The average of 24 readings showed an increase in threshold of perception from 19 volts before alcohol to 30 volts after alcohol, and with pain from 82 volts before alcohol to 110 volts after alcohol. The subjects were quite cheerful about taking an amount of faradic stimulation under alcohol that they would not tolerate before alcohol, and after alcohol they even laughed and joked about the strong current.

Respiration was much slower after alcohol and there was sometimes Cheyne-Stokes breathing. The respiratory cr disappeared after alcohol.

Effect on *crs*. The *motor reactivity* was increased in amplitude, far beyond the needs of the reaction and hence could be considered a loss of differentiation or adaptation. The *retention* of previously formed

excitatory crs was disturbed but not nearly as much as the differentiation between the excitatory and inhibitory conditional stimuli. The subject frequently reacted to the inhibitory conditional stimuli as if they were excitatory but did not make the opposite mistake of failing to react to the excitatory conditional stimuli until the later stages of the alcoholic effect. The ability to form new crs under the influence of alcohol was much more impaired than was the retention of the previously formed crs. This is in keeping with the observations of one of us (16) that retention is a more stable function of the cortex than is the ability to form new conditional reflexes; the latter function is one of the first to disappear in an organic or toxic agent acting on the cortex. Though the integrated crs suffered after alcohol, a subcortical UR, viz., the orienting reflex, which is usually in abeyance after learning has occurred, became prominent. This reappearance of the formerly suppressed orienting reflex was noted in dogs (18, p. 67) as well as in humans after alcohol.

Not only was there impairment of differentiation in the crs, but there appeared another abnormality showing lack of inhibition, viz., the appearance of the primary cr which was not present before alcohol. This consists in removing the hand from the electrode instead of using in response to the red light the indirect method of avoiding the shock by throwing a switch or pressing a bulb. The primary cr is seen in some patients as well as in most young children and dogs (Fig. 2, top line).

Subjectively all of the subjects were more talkative, cheerful, often loud and boisterous, later somnolent, after alcohol. Their statements indicated less concern about giving the correct responses, e.g., "I don't care whether I do it or not, I feel fine", "I felt that what I was going to get (shock) was not worth bothering about", "I don't give a damn how I do this". The dependence of the cr upon the intensity of the unconditional stimulus (motivation) was shown in the statements of one of the subjects—"If it had been a heavier shock I might have fought more".

It is an interesting comment that in spite of the superior knowledge of the medical students used in the test they were unable to appraise subjectively their performance under alcohol, even the next day when they were no longer under its influence. This was in contrast to the fact that the subjects often recognized during the test immediately

after he had reacted to an inhibitory stimulus that he had made a mistake; but it seems that the motor cr to the inhibitory stimulus always peceded its evaluation by the subject.

In comparing the effect of benzedrine and alcohol on the sexual reflexes in dogs it was stated that benzedrine increased the latent period of the sexual reflexes, i.e., delayed ejaculation. Alcohol was found to have a similar though more pronounced effect (19). The explanation may rest upon the diminished sensitivity after alcohol, an effect shown in our present experiments as well as by H. G. Wolff and others.

In the number completion of the Army Alpha test one subject continued to make a perfect score while under alcohol but whereas his speed increased with repetition (plus 20 per cent) in the control test, it decreased (minus 45 per cent) after alcohol. In the verbal reports

TABLE 4

Results after the administration of alcohol to four subjects to demonstrate sensitivity of conditioned reflex test

	RESTING RESP. PER MIN.	LATENT PERIOD MOTOR RESPONSE	PER CENT POSITIVE CRS CORRECT	PER CENT NEG. CRS CORRECT	CONDITIONED RESP. REFLEX
Control readings	21	1.0	97%	90%	Present
Readings after 2 cc./kg. alcohol	10	1.6	88%	50%	Absent

of the subjects after alcohol there was no difference that could be clearly expressed by a score; by the method of grading used the difference is negligible.

While it is true that even so complex a function as language is capable of analysis and may even obey mathematical laws, as shown by Whitehorn (20) and others, nevertheless the difficulty of its evaluation and the variation of its symbolic uses in the same individual at different times and in different individuals often vitiate conclusions drawn from tests in which language is chiefly employed.

It was thus apparent that the cr method was adequate to demonstrate changes in the ability to differentiate, changes in the latent period of reaction, and changes in the respiratory cr after a drug such as alcohol, and that the effect of a large dose of amphetamine sulfate was not comparable in its disturbing effect to that of a large dose of alcohol.

DISCUSSION

The increase of blood pressure and respiratory rate after the administration of amphetamine sulfate indicated that the drug had been absorbed from the intestinal tract. Any difference between the control and drug series could be attributed to the drug, as other factors had been eliminated by the first controls. Increase in blood pressure in the absence of change in pulse rate must be due either to an increased stroke volume of the heart or to peripheral vasoconstriction. The general sympathomimetic action of the drug favors the latter explanation.

In contrast to alcohol, there was with benzedrine no influence on the peripheral sense organs as judged by the threshold of sensitivity to an electrical shock and acuity of hearing. A slight effect on the cerebral cortical activity as measured by the motor cr was noted, but this was limited to increasing the number of correct responses to a negative conditional stimulus, i.e., the subject less frequently threw the cutoff switch after a stimulus which was not followed by an electrical shock. No change was seen in the number of correct responses to a positive conditional stimulus nor in the latent period of the response. Apparently there was a slight improvement in the ability of the subject to differentiate between the positive and negative stimuli, but there was no stimulation of cr activity in the sense of increased intensity of reaction. This result was contrary to that obtained with dogs, in which there was some stimulation and a slight loss of differentiation. Although this difference may have been a species variation in response, it was more probably due to the difference in dose, since the dogs received 1.0 mgs. per kg. body weight, but the human subjects less than 0.4 mg. per kg. Whether comparable doses would produce loss of differentiation in human beings has not been determined, since the higher dosage is beyond the usual range of therapeutic dosage for patients (5–30 mgs.), and was not administered for fear it might have a toxic effect.

Unlike the cr test, the number series test was unable to pick up any difference in performance after the administration of amphetamine sulfate, and very little even after 2.0 cc. of alcohol per kg. This indicates that the conditional reflex test is a more sensitive and easily appraised indicator of cerebral cortical activity; furthermore it was

more readily interpreted and the motivation (intensity shock) could be measured. In both of these tests there was a wide range in the results from different individuals though their educational training was similar. This variability in the response to amphetamine—a characteristic of the drug—requires that the apparent results of drug administration be carefully analyzed.

The most striking fact was that with the general improvement in mood, there was no impairment of performance (unlike alcohol) but even a slight improvement (as measured by cr activity in human beings) with doses up to 30 mg. of amphetamine sulfate. The current use of this drug by armed forces under periods of stress gives a special significance to this fact.

We are indebted to Drs. J. W. T. Redfern and John Butterfield for assistance in these experiments.

SUMMARY

1. Amphetamine sulfate in the oral dosage of 10–15 and 20–30 mgs. increased the systolic and the diastolic blood pressure of healthy young adults but did not alter the pulse rate.

2. The resting respiratory rate was increased.

3. The drug had an effect on motor conditional reflexes, viz., slight improvement in differentiation between positive and negative conditional stimuli.

4. The threshold of sensation to an electrical shock, the acuity of hearing, and the performance with a number series problem test were unchanged by amphetamine.

5. The presence of a respiratory cr in humans was observed.

6. In comparison with amphetamine, alcohol (1.5 cc./kg.) had a pronounced effect on motor crs. Alcohol favors excitatory reactions, interferes with inhibition, tends to convert inhibitory into excitatory reactions, impairs the respiratory cr, and markedly increases the threshold for perception and for pain.

7. The cr test was more sensitive in detecting a change in higher nervous function than were the verbal tests which were used.

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CLINICAL AND PATHOLOGICAL FINDINGS IN AORTIC ATRESIA OR MARKED HYPOPLASIA OF THE AORTA AT ITS BASE

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Atresia or marked hypoplasia of the aorta at its base, although a rare malformation, is a well recognized pathological entity. The following two cases show that this malformation produces a distinctive clinical picture.

CASE REPORTS

Case 1. R. S. (HLH # A6841) a white male infant who was brought to the Harriet Lane Home when 36 hours of age because of intense cyanosis.

Physical examination: Temperature 37°. Weight 3.5 kilograms. The infant was gasping for breath. The heart was enlarged; the rate was rapid; there were no murmurs. The pulse was weak, but palpable in both the arm and the leg. The lungs were clear. The liver was engorged, but did not pulsate. The extremities showed pronounced cyanosis.

Fluoroscopic examination showed that the heart was enormous. In the A-P view the right ventricle was seen to be greatly enlarged; the pulmonary conus of the right ventricle was abnormally full. Due to the dilatation of the superior vena cava the shadow cast by the great vessels to the right of the sternum appeared abnormally wide. In the left anterior oblique position, the right ventricle extended nearly to the anterior chest wall.

Laboratory data: Red blood count was 5,650,000. Hemoglobin was 160%. The electrocardiogram, taken when the patient was moribund, showed a slow idioventricular rate of 30, great widening of the QRS complex and a right axis deviation.

Clinical impression: Congenital malformation of the heart of the cyanotic group. The marked enlargement of the right ventricle indicated that it was the chamber of functional importance. Further-

more, the huge size of the heart at two days of age indicated that the heart had been under great strain during fetal life. The dilatation of the pulmonary conus and the pulmonary artery showed that that vessel occupied its normal position. This finding indicated that the blood from the right ventricle was being pumped into the pulmonary artery in the normal manner. Inasmuch as the right ventricle was the chamber of functional importance and the blood was being pumped out into a normal pulmonary artery, it was deduced that the blood to the systemic circulation was being pumped from the right ventricle through the ductus arteriosus to the aorta. For this to be possible, there must be atresia of the aorta at its base. The dilatation of the superior vena cava suggested the possibility of a gross defect in the interauricular septum of such a nature as to make the two auricles function as a single chamber.

Autopsy # 15868 (performed by Dr. Mark) showed the following positive findings: The heart was greatly enlarged. The blood from the superior vena cava and the inferior vena cava entered normally into the right auricle. The foramen ovale was widely patent and only partly protected by a valve-like membrane; the opening in the upper portion of the foramen ovale measured 1 cm. in diameter. The opening was so large that it seemed as if most of the interauricular septum was lacking; its upper margin was represented only by a narrow band of tissue. The tricuspid valve was normal and opened into a greatly enlarged right ventricle. The wall measured 0.7 cm. in thickness. Posterior to the tricuspid valve there was a small defect at the base of the right ventricle above which the diminutive aorta arose. The pulmonary conus of the right ventricle was markedly enlarged; the pulmonary valve was normal. The pulmonary artery arose from the right ventricle in the normal manner; it was a thin-walled wide vessel which measured 3 cm. in circumference. The pulmonary artery gave off its two branches to the lungs and then it continued as a wide channel (6–7 mm. in transverse diameter) which opened directly into the aorta. The relation of this channel to the two main branches of the pulmonary artery and to the aorta showed that it was the ductus arteriosus. The pulmonary veins opened into the left auricle in the normal fashion. The mitral valve was normal. The left ventricle was small. At the base of the membranous portion of the interventricular septum there

was a small opening between the ventricles. As previously mentioned, the orifice of the aorta lay above the upper margin of the septal defect; it could receive a small quantity of blood from both ventricles. The valves of the aortic orifice were fused together forming a cone-like tube with a small opening. The aorta, itself, was a diminutive thin-walled vessel. The coronary arteries arose normally from the base of the aorta. The aorta gave off three large vessels to the head and upper extremities and then remained narrow until it reached the point of

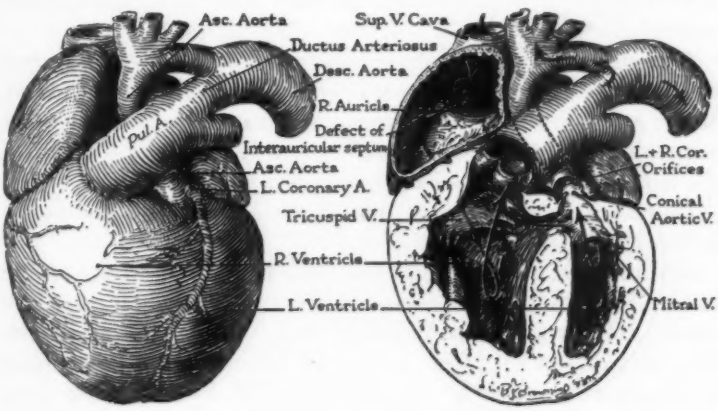

FIG. 1. Case 1: Extreme aortic stenosis and hypoplasia of the ascending aorta, with under-development of the left ventricle, and a high ventricular septal defect. There is also a gross defect in the interauricular septum. (Drawing by Miss Betty Jane Browning.)

entrance of the abnormally wide ductus arteriosus; thereafter the aorta was normal in appearance. Figure #1 shows a drawing of this specimen.[1] In addition there were numerous fenestrations in the musculature of the interventricular septum measuring 3–4 cm. in diameter which were apparent only when the wall was distended.

Anatomical diagnosis: Congenital malformation of the heart. Cardiac hypertrophy with enlargement of the right side. Widely patent foramen ovale and multiple minute interventricular septal defects.

[1] Unfortunately this specimen was lost before the drawing was completed; therefore it is not exact in every detail.

Fused aortic cusps. Small aorta arising above the upper margin of the interventricular septal defect. Widely patent ductus arteriosus.

Case 2. Baby G., born on the Johns Hopkins Obstetrical Service. At three days of age he became intensely cyanotic.

Physical examination showed an intensely cyanotic baby whose respirations were very rapid. The heart rate was rapid. There was a definite systolic murmur over the precordium. The pulse was almost imperceptible. The lungs were clear. The liver extended to the umbilicus. Cyanosis was of equal intensity in the upper and the lower extremities.

Fluoroscopic examination showed that the heart was greatly enlarged and that the enlargement was mainly of the right side of the heart. In the A-P view the pulmonary conus was markedly distended; in the left anterior oblique position the right ventricle was huge. In addition the right auricle and the superior vena cava were dilated.

Electrocardiogram showed a normal sinus mechanism and a pronounced right axis deviation.

Clinical diagnosis: Congenital malformation of the heart and cardiac failure. The intense cyanosis and the early evidence of cardiac failure showed the condition was incompatible with life. The great right-sided enlargement indicated that the right ventricle was the chamber of functional importance. The dilatation of the pulmonary conus indicated that the pulmonary artery was the vessel of functional importance. From these findings it was deduced that there was aortic atresia. Death occurred at five days of age.

Autopsy # 16929 (performed by Dr. Wagley) showed the following positive findings: The heart was moderately enlarged. The right auricle and the right ventricle were greatly dilated and hypertrophied. The superior vena cava and inferior vena cava entered the right auricle in the normal manner. The foramen ovale was patent but not large. The tricuspid valve was normal and opened into a greatly dilated and hypertrophied right ventricle. The pulmonary valve was normal. The ductus arteriosus was widely patent and extended from the bifurcation of the pulmonary artery to the aorta, into which it opened at the usual point. Beyond the entrance of the ductus arteriosus the aorta was normal. Proximal to the ductus arteriosus, the aorta was small. The transverse arch of the aorta was smaller in width than

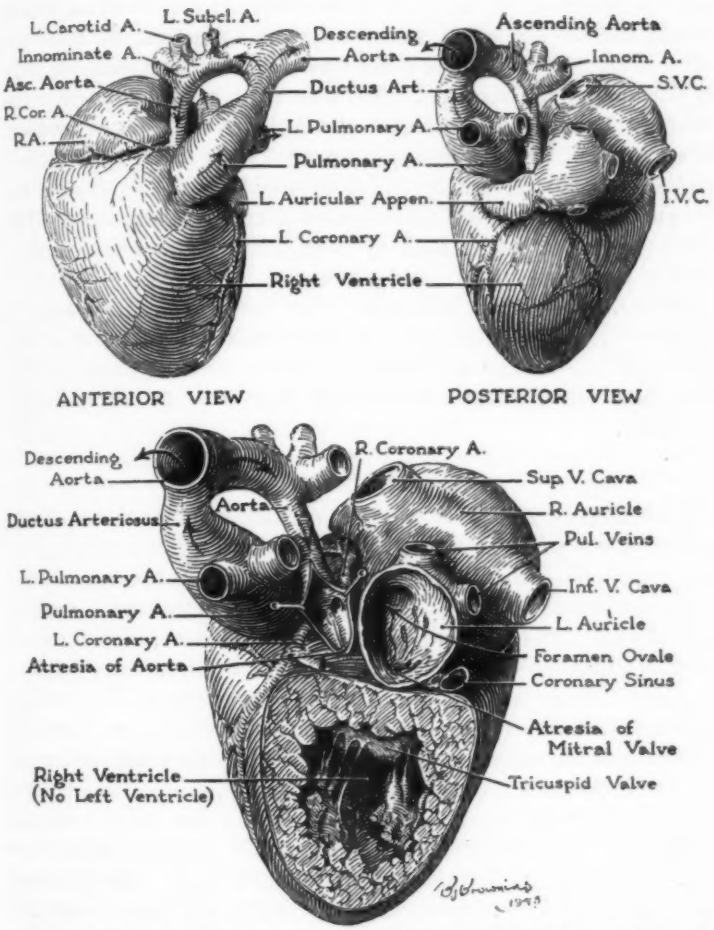

FIG. 2. Case 2: Aortic atresia and marked hypoplasia of the ascending aorta. Upper drawings show the external appearance of the heart. Distortion of the heart upon fixation is due to the absence of the left ventricle. In the lower drawing the posterior wall is cut away to show the mitral atresia and aortic atresia. (Drawing by Miss Betty Jane Browning.)

was the ductus arteriosus. The innominate artery, the left common carotid and the left subclavian arose in the normal fashion from the aortic arch. The ascending aorta was very small and ended blindly at the base of the ventricle. Just above the base of the aorta the coronary arteries were given off the aorta in the normal manner. There were but three pulmonary veins; all of these entered into the left auricle in the normal fashion. The only way for the blood to leave the left auricle was through the foramen ovale to the right auricle. The mitral valve was represented by a puckered scar. There was no trace of the left ventricle (see figure 2).

Anatomical diagnosis: Triloculate heart, two auricles, and a single ventricle—the right ventricle. Mitral atresia with absence of the left ventricle. Atresia of the aortic orifice. Absence of one of the pulmonary veins, patent foramen ovale and a widely patent ductus arteriosus.

DISCUSSION

The clinical findings in the two cases were remarkably similar. Both infants were cyanotic at birth and showed intense cyanosis throughout their brief lives. Within the first few days of life both showed enormous cardiac enlargement and evidence of cardiac failure with marked engorgement of the liver and an extremely weak pulse. Upon fluoroscopic examination[2] in both instances it was evident that the enlargement of the heart was primarily due to the great enlargement of the right auricle and the right ventricle. In the A-P view the heart was enlarged and the pulmonary conus was markedly distended; this indicated that the pulmonary artery was normally placed. Examination in the left anterior oblique position showed that the anterior margin of the cardiac shadow extended forward nearly to the anterior chest wall. This finding confirmed the impression that the right ventricle was huge. The posterior margin of the cardiac silhouette did not extend further back than normal, showing the left ventricle was not enlarged.

These findings indicated that the right ventricle was doing the main work of the heart. The fact that the pulmonary conus was in the

[2] X-rays were not obtained in either case due to the critical condition of the patients.

normal position and markedly dilated indicated that the pulmonary artery was given off the right ventricle. Therefore, it could be deduced that the blood to the sytemic circulation was being pumped from the right ventricle by way of the pulmonary artery; hence the blood must flow from the pulmonary artery through the ductus arteriosus to the aorta. For this to be possible the pressure in the pulmonary artery must be higher than the pressure in the aorta. In other words little, if any, blood could reach the aorta through its normal channel. The most probable cause of such a condition would be complete or functional atresia of the aortic orifice.

At autopsy both cases showed the ascending aorta and transverse arch were diminutive in size and there was marked enlargement of the right ventricle, an enlarged pulmonary artery and a widely patent ductus arteriosus. In the first case the aorta was a diminutive tube which could receive a small quantity of blood from the thin-walled left ventricle and also from the right ventricle. In the second case there was complete atresia of the aortic orifice, mitral atresia, and the left ventricle was non-existent. In both the right auricle and right ventricle were huge and the pulmonary artery occupied its normal position. The ductus arteriosus was widely patent and the main blood supply to the systemic circulation was from the pulmonary artery through the ductus arteriosus to the aorta. In both the aorta remained as a diminutive tube; the coronary arteries arose in the normal fashion from the root of the aorta.

Thus the autopsies confirmed the clinical diagnosis and showed that the reasoning was sound.

SUMMARY

Aortic atresia or marked hypoplasia of the aorta at its base causes the right ventricle to pump the blood not only to the lungs but also through the ductus arteriosus to the systemic circulation. This mechanism places a great strain on the right side of the heart and is extremely inefficient for the maintenance of an adequate circulation to the body.

The outstanding clinical features produced by this malformation are intense cyanosis, great right-sided cardiac enlargement, a weak pulse in both the arms and the legs and a low blood pressure. Fluoro-

scopic examination shows cardiac enlargement due to the enormous enlargement of the right ventricle; the pulmonary conus of the right ventricle and the pulmonary artery proximal to the ductus arteriosus occupy their normal position but are markedly distended. Usually there is also distention of the right auricle and the superior vena cava. The electrocardiogram shows a right axis deviation. Cardiac failure occurs early. The condition is not compatible with life for more than a few days.

THE METHYLATION OF NICOTINAMIDE

A Simple and Practical Test for Liver Function[1] (A Preliminary Note)

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The study of the methylation mechanism in the body under various physiological conditions, as well as in disease, has been hampered largely by the lack of a suitable and simple method of approach. The need for a satisfactory and convenient method for such an investigation is therefore obvious.

In 1940 Najjar and Wood (1) reported the presence in urine of a nicotinic acid derivative which possessed a bluish fluorescence in ultraviolet light when treated with alkali and butanol. This fluorescent property formed the basis of a simple method of measuring the compound. The excretion of this substance, subsequently designated F_2 (2), was shown to be absent in nicotinic acid deficiency and to vary directly with the intake of nicotinic acid or nicotinamide (3, 4). This fact led successfully to the establishment of a load test for the diagnosis of nicotinic acid deficiency (2–5) inasmuch as the deficient organism excreted in the urine significantly smaller amounts of F_2 compared to normal.

Chemical studies by Huff and Perlzweig (6, 7), Najjar et al. (8–10) and Ellinger and Coulson (11, 12) have shown that the F_2 compound excreted in urine after the ingestion of nicotinamide is a methylated nicotinamide where the methyl group is attached to the ring nitrogen,—N^1-methylnicotinamide (F_2). Thus a significant amount of the ingested vitamin is methylated in the body and excreted as such in the urine. It occurred to us that, aside from its established value as a means of diagnosing nicotinic acid deficiency, this might form a simple tool for the study of methylation in the non-deficient individual. Therefore, in states of adequate nutrition, measurement of the extent of methylation of a given dose of nicotinamide could afford an indica-

[1] This study was aided by a grant from Mead Johnson and Company.

tion of the behaviour of this mechanism under various conditions, physiological as well as pathological.

The fact that methylation is known to occur in the liver (13) and the finding that liver tissue (8) is particularly rich in F_2 (N^1-methylnicotinamide) suggest that methylation of nicotinamide might also take place in that organ. Perlzweig et al. (14), using rat tissue slices, showed that nicotinamide is methylated *in vitro* by liver but not by kidney or muscle tissue. The possibility that this might also be the case *in vivo* was suggested by our preliminary studies on patients with liver disease, which showed that after a load test with nicotinamide only a small fraction of the dose was methylated compared to normal. It became necessary, therefore, to investigate that possibility and determine the site of methylation *in vivo* by the infusion of the individual intact organ with nicotinamide solution.

EXPERIMENTAL

A dog weighing approximately twenty pounds was anesthetized with ether. The abdominal cavity was opened and widely exposed. The liver and left kidney were isolated *in vivo* in the following manner:

The right kidney was severed and served as a control. The left kidney was shelled out of its capsule and brought out of the abdominal cavity, leaving its arterial supply and venous drainage intact. The ureter was dissected out, severed at its junction with the bladder and allowed to drain into a test tube. The renal vein was then clamped and a 25 cc. control sample of blood was obtained from the vein on the renal side of the clamp. 50 mg. nicotinamide were then injected into the renal artery during a period of about two minutes while blood was simultaneously being withdrawn from the vein in order to establish a continuous blood flow, as well as obtain blood samples. The kidney was then completely severed and weighed slices used for assay.

The liver was detached from the surrounding organs to eliminate any aberrant blood supply. While it remained, of necessity, in its natural location, its only connection with the abdominal cavity was with the hepatic vessels, portal vein and hepatic ducts. A piece of liver tissue and a sample of blood from the hepatic vein were obtained as control specimens. The hepatic artery and portal vein were then simultaneously clamped. 50 mg. nicotinamide were immediately injected

into the portal vein on the hepatic side of the clamp, while samples of blood were being withdrawn from the hepatic vein. With this procedure there was no possibility that the nicotinamide injected could be methylated elsewhere and return as such to the liver, since all blood supply was cut off. A piece of liver tissue was removed during the injection which lasted about three minutes and three other pieces were cut out at approximately one minute intervals after the injection.

Samples of the tissues obtained were thoroughly ground and extracted with acetate buffer over a steam bath for twenty minutes.

TABLE I

The Methylation, in vivo, of Nicotinamide by the Kidney and Liver of the Dog
(50 mg. injected into the renal artery and portal vein)

Micrograms of Methylated Nicotinamide (F_2) Expressed as N^1-Methylnicotinamide Chloride

	CONTROL SAMPLES	NICOTINAMIDE INFUSED SAMPLES	REMARKS
	γ per gram	*γ per gram*	
Kidney	0.98–1.3	0.92–1.5	After Injection
Liver	0.90–0.95	3.8	During Injection
		4.4	1 Min. After Injection
		4.1	2 Min. After Injection
		3.8	3 Min. After Injection
	γ per 100 cc. serum	*γ per 100 cc. serum*	
Blood obtained from the renal vein	traces (not measurable)	traces (not measurable)	During Injection
Blood obtained from the hepatic vein	69	108	During Injection

Blood serum was added to acetate buffer and likewise steamed and extracted. All extracts were assayed for N^1-methylnicotinamide (F_2) by the method of Najjar (15).

The results (Table I) show that the intact kidney was incapable of methylating nicotinamide. There was no demonstrable increase of the methylated compound, neither in the blood nor in the renal tissue after infusion with nicotinamide. Futhermore, the urine collected from the infused kidney showed no increase in N^1-methylnicotinamide. On the other hand, the N^1-methylnicotinamide content of liver tissue rose promptly during the injection, as well as

after its termination, to values four to five times that of the control.

It is then obvious that of the two organs the liver is the one responsible for the methylation of nicotinamide. This, of course, does not indicate that the liver is the only organ responsible for this mechanism. However, from the data obtained, the total amount of nicotinamide that was methylated by the whole liver was found to be nearly three percent of the injected dose; a very substantial amount considering the short time allowed by this experiment and that nicotinamide passed only once through liver tissue. Moreover, this does not include the quantity that was methylated and escaped through the hepatic vein as shown by the rise in F_2 blood levels during the infusion. By comparison, in the normal dog, where methylation is not interrupted, the total amount methylated and excreted after the same dose is about six to seven per cent.

The agreement between our results *in vivo* and those of Perlzweig et al. (14) *in vitro* with regard to the liver and kidney, makes it likely that muscle tissue, which was found incapable of such methylation *in vitro*, is similarly inactive in the live animal.

METHYLATION IN NORMAL SUBJECTS

Six adults were used for this experiment. Four ingested 500 mg. nicotinamide[2] daily for a period of eight to twelve days. Two others were put on an intake of 300 mg. for sixteen days. The output of the methylated nicotinamide was followed each day. It was found that after a preliminary rise, the output was not maintained at a high constant level but showed a steady decline. Figure 1 shows the excretion of one subject on a 300 mg. intake and represents the typical pattern of excretion exhibited by the other subjects. The results seem to indicate that the methylating process, occurring mainly if not all in the liver, might be overburdened and depleted of its capacity to cope with a persistent demand for methylation. That such a process might take place, with pathological manifestation in the liver, is strongly indicated by the work of Handler and Dann (16). They showed that with large doses of nicotinamide rats develop fatty livers. All this does not necessarily imply that other adjustments in the body or in the

[2] Generously supplied by Merck and Company, Inc.

methylating system do not take place. Such adjustments, if any, would tend towards an overall economy to spare the methyl radical.

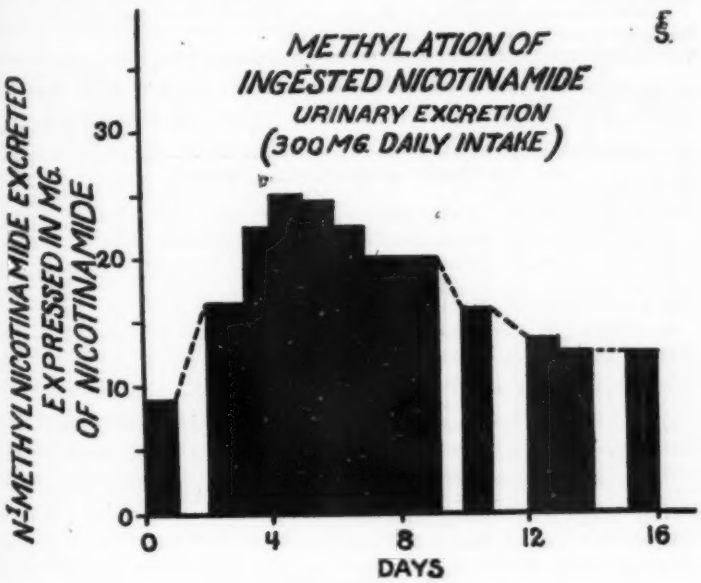

FIG. 1

This, however, does not appear very likely although such a possibility demands further investigation.

METHYLATION IN DISEASE

If the methylation of nicotinamide is a function of the liver mainly if not altogether, one could reasonably expect that diseases related to or affecting the function of that organ would cause a reduction of the capacity to methylate the vitamin.

A number of patients, between the ages of three and fourteen years, suffering from various diseases were investigated to ascertain this point. None of the patients presented any signs or gave a dietary history suggestive of nicotinic acid deficiency. The ability of the patient to methylate was studied by measuring the urinary output of the methylated nicotinamide during a four-hour period following a

TABLE II

Methylation in Various Diseases

Test Doses of Nicotinamide (50 mg. Orally and 20 mg. Intravenously)

Figures represent percentage of the test dose methylated and excreted in the urine during the first four hours after the dose was given.

PATIENT	AGE	SEX	DIAGNOSIS	PER CENT METHYLATED	
				i.v. dose 20 mg.	oral dose 50 mg.
			(Diseases not related to liver function)		
E. K.	4	F.	Infectious Polyneuritis		3.8
G. N.	3	F.	Pneumonia, Congenital Heart Disease		6.0
G. Y.	3	M.	Peritonitis		3.0
N. H.	9	F.	Cystitis	5.0	3.0
B. B.	7	F.	Bronchiectasis	8.4	3.7
W. B.	8	M.	Rheumatoid Arthritis	7.5	3.4
W. D.	10	M.	Rheumatoid Arthritis	5.0	2.9
A. V.	4	M.	Rectal Polyp		5.2
D. M.	9	F.	Rectal Polyp		7.5
J. B.	5	M.	Rheumatic Fever	11.3	
H. J.	8	F.	Rheumatic Fever	6.8	3.3
W. R.	10	M.	Rheumatic Fever	7.9	4.9
B. T.	5	F.	Rheumatic Fever with Sickle Cell Anemia	12.5	5.5
D. M.	13	F.	Rheumatic Fever	6.1	
M. D.	7	M.	Rheumatic Fever	11.2	4.4
			(Diseases related to liver function)		
V. S.	3	M.	Nephrosis	4.5	5.6
S. M.	3	F.	Nephrosis	4.5	3.2
E. B.	4	F.	Nephrosis	(1) 1.1 (2) 2.4	(1) 0.6 (2) 1.1
B. W.	7	M.	Diabetes Mellitus		5.9
L. R.	11	F.	Diabetes Mellitus		2.4
T. B.	8	M.	Diabetes Mellitus		1.9
M. H.	13	F.	Diabetes Mellitus	3.5	2.2
G. L.	14	M.	Diabetes Mellitus	4.2	1.4
S. S.	8	F.	Catarrhal Jaundice (Convalescent)		1.9
H. B.	11	M.	Catarrhal Jaundice (Convalescent)		1.0
W. M.	3	F.	Glycogen Disease		0.8
P. M.	6	M.	Glycogen Disease		(1) 0.1
*P. M.	9			1.3	(2) 0.6
R. E.	3	M.	Glycogen Disease		1.1

* P. M.—Two load tests were done three years apart.

load test of either 50 mg. nicotinamide by mouth or 20 mg. injected intravenously.[3] Whenever possible both load tests were done on the same patient with an interval of three to four days between the tests. Table II shows the results of the load tests.

It is apparent from the results that there is a close parallelism between the oral and the intravenous load tests in all patients studied. Both tests showed that patients with no liver involvement possessed a greater capacity to methylate than those suffering from diseases involving the liver. In every case there was a higher degree of methylation with the intravenous than with the oral test. This is probably due to mass action incident to the higher levels obtained with the intravenous route.

The most striking results are those obtained on cases with glycogen disease. This is not surprising as liver function in this disease is in many ways impaired. All three patients showed a very low output of N^1-methylnicotinamide. Patient P. M. showed a negligible degree of methylation at the age of six and when tested about three years later still showed very marked impairment. The two cases of catarrhal jaundice,[4] studied during convalescence, likewise showed marked reduction in their methylating capacity. Of the three nephrotics, one (E. B.) showed marked diminution of response in repeated tests.[5] Of the five diabetics studied only one showed values comparable to those of the control cases. The other four had values that are somewhat low.

DISCUSSION

We have shown above that in the experimental dog the infusion of the kidney with a nicotinamide solution produces no increase in its

[3] A control four-hour urine specimen was collected after an eight-hour fast. On the following day a load test was given and a four-hour urinary collection was made thereafter. Both urine collections, that of the control and the load test, were made from twelve midnight to four A.M. Specimens were preserved by adding to the urine enough glacial acetic acid to make a three percent concentration of the acid. Assays were carried out according to the procedure of Najjar (15).

[4] These two cases were studied after their urine became clear of bile pigment.

[5] The possibility that E. B. might have failed to excrete the methylated nicotinamide in four hours was ruled out by the demonstration that subsequent collection following the first four hours of the load test failed to show values above her usual fasting levels.

content of methylated nicotinamide. Neither the blood flowing out of the infused kidney, nor the urine filtering through it, showed any measurable increase. This organ, therefore, plays no part in the methylation of nicotinamide. By contrast, the infused liver showed an immediate and marked increase in N^1-methylnicotinamide (F_1), in the liver tissue as well as in the blood draining therefrom. The capacity of the liver to methylate the vitamin seems to be remarkably large. It could very well account for the total methylation of nicotinamide in the body. Experiments, *in vitro*, on rat liver slices (14), as well as the production of fatty liver in rats by feeding large doses of nicotinamide (16) thereby overtaxing methylation in that organ, all tend to support this view. The results of feeding relatively large doses to human subjects over a period of time (Figure 1) suggest that in man, as in the rat, the methylation process in the liver is apt to manifest signs of impairment and exhaustion.

Our observations with the use of a nicotinamide load test on patients with liver disease, although admittedly few, are sufficiently striking to warrant further pursuance of this work. The results on glycogen disease, if confirmed on a larger series than we have, would be of particular interest and afford ample room for speculation. The defect, if such is the case, is apparently a permanent one since patient P. M. manifested the same impairment of methylation at the age of six years and again three years later at the age of nine. That such a defect also existed earlier in his life is suggested by a similar impairment in the other two cases, W. M. and R. E., both three years old. Whether methionine, choline and methyltestosterone can enhance methylation of nicotinamide in general and in these cases in particular, remains to be seen. This problem is under investigation.

The load test with nicotinamide has the particular advantage that the test substance is a vitamin and is not apt to induce other than physiological responses from the body. The methylated product is likewise physiological, since it is found normally in tissues and apparently possesses biological properties (17). In nutritional deficiency the load test with nicotinamide has its value as a measure of nicotinic acid deficiency and in such states should not be interpreted as a measure of methylation.

SUMMARY

1. The liver in the dog is capable of methylating nicotinamide to an appreciable degree *in vivo*. However, the kidney is incapable of such methylation.

2. Patients with diseases affecting the liver appear to show a diminution of their capacity to methylate a given dose of nicotinamide.

3. It is recommended that a load test of nicotinamide, given either orally or intravenously, preferably the latter, be used as a liver function test for the study of the methylating mechanism under various physiological conditions and in disease.

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